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Impact of long-term intravitreal anti-vascular endothelial growth factor on preexisting microstructural alterations in diabetic macular edema

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Abstract: **PURPOSE:** Evaluation of the influence of long-term intravitreal anti-vascular endothelial growth factor treatment on preexisting retinal microstructural alterations in patients with diabetic macular edema. **METHODS:** Eyes with diabetic macular edema and a history of 20 intravitreal anti-vascular endothelial growth factor (aflibercept and/or ranibizumab) injections were included in this retrospective study. Primary outcome was the extent of disorganization of retinal inner layers, alterations at the outer plexiform layer/Henle fiber layer junction, disruption of external limiting membrane/ellipsoid zone, disruption of retinal pigment epithelium/Bruch complex, and retinal atrophy at baseline versus after 20 intravitreal injections as visualized by spectral-domain optical coherence tomography images. **RESULTS:** Of 383 eyes screened, 37 eyes were included in the current study. With the exception of outer plexiform layer/Henle fiber layer junction restoration, no significant changes regarding microstructural alterations between baseline and end of study were encountered after long-term anti-vascular endothelial growth factor (disorganization of retinal inner layers $P = 0.381$, outer plexiform layer/Henle fiber layer junction $P = 0.001$, external limiting membrane/ellipsoid zone $P = 0.524$, retinal pigment epithelium/Bruch complex $P = 0.122$, retinal atrophy $P = 0.317$). Best-corrected visual acuity significantly increased over the course of the study, corresponding to central retinal thickness and intraretinal fluid reduction (all $P < 0.0001$). The extent of microstructural alterations was negatively correlated with best-corrected visual acuity ($P < 0.05$). **CONCLUSION:** Apart from outer plexiform layer/Henle fiber layer junction layer restoration, no effect on preexisting retinal alterations was encountered after long-term intravitreal injections. Thus, intravitreal ranibizumab or aflibercept did not have a major effect (neither positive nor negative) on microstructural alterations.

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IMPACT OF LONG-TERM INTRAVITREAL ANTI-VASCULAR ENDOTHELIAL GROWTH FACTOR ON PREEXISTING MICROSTRUCTURAL ALTERATIONS IN DIABETIC MACULAR EDEMA

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Purpose: Evaluation of the influence of long-term intravitreal anti-vascular endothelial growth factor treatment on preexisting retinal microstructural alterations in patients with diabetic macular edema.

Methods: Eyes with diabetic macular edema and a history of ≥ 20 intravitreal anti-vascular endothelial growth factor (aflibercept and/or ranibizumab) injections were included in this retrospective study. Primary outcome was the extent of disorganization of retinal inner layers, alterations at the outer plexiform layer/Henle fiber layer junction, disruption of external limiting membrane/ellipsoid zone, disruption of retinal pigment epithelium/Bruch complex, and retinal atrophy at baseline versus after ≥ 20 intravitreal injections as visualized by spectral-domain optical coherence tomography images.

Results: Of 383 eyes screened, 37 eyes were included in the current study. With the exception of outer plexiform layer/Henle fiber layer junction restoration, no significant changes regarding microstructural alterations between baseline and end of study were encountered after long-term anti-vascular endothelial growth factor (disorganization of retinal inner layers $P = 0.381$, outer plexiform layer/Henle fiber layer junction $P = 0.001$, external limiting membrane/ellipsoid zone $P = 0.524$, retinal pigment epithelium/Bruch complex $P = 0.122$, retinal atrophy $P = 0.317$). Best-corrected visual acuity significantly increased over the course of the study, corresponding to central retinal thickness and intraretinal fluid reduction (all $P < 0.0001$). The extent of microstructural alterations was negatively correlated with best-corrected visual acuity ($P < 0.05$).

Conclusion: Apart from outer plexiform layer/Henle fiber layer junction layer restoration, no effect on preexisting retinal alterations was encountered after long-term intravitreal injections. Thus, intravitreal ranibizumab or aflibercept did not have a major effect (neither positive nor negative) on microstructural alterations.

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Diabetic macular edema (DME) represents the major cause of visual impairment among diabetic patients, occurring in approximately 35% of patients with diabetic retinopathy.^{1,2} Complex biochemical pathways lead to microvascular dysfunction, increased local inflammation and oxidative stress, resulting in the accumulation of cytokines and growth factors. This results in a breakdown of the blood–retinal barrier and an accumulation of intraretinal fluid (IRF).³ The most important alterations at the blood–retinal barrier include pericyte loss, thickening

of the basement membrane, and decreased intercellular connections.⁴ Changes at the level of the photoreceptors and the retinal pigment epithelium (RPE) occur due to an accumulation of reactive oxygen species and the impairment of vascular integrity.³ In addition to the described vascular changes, alterations at the neurovascular unit (Müller cells, astrocytes, ganglion cells, amacrine cells, retinal vascular endothelial cells, and pericytes) play a role in the pathogenesis of DME.⁵ Recent reports consider diabetic retinopathy as neurodegenerative disease.⁶

Current treatment strategies for DME include intravitreal anti-vascular endothelial growth factor (anti-VEGF) agents, intravitreal steroid injections, and grid laser photocoagulation. At present, anti-VEGF agents represent the first-line therapy option.^{7,8}

Because DME mostly requires long-term treatment and is often started rather early in life, the impact of multiple intravitreal anti-VEGF injections on the retinal microstructure is of great importance. A progression of atrophic changes after long-term anti-VEGF exposure in age-related macular degeneration was recently reported by Schuetze et al.⁹ However, age-related macular degeneration represents a disease with preexisting RPE damage, comparison with DME may therefore not be appropriate.^{10,11} Up to now, only a limited amount of reports regarding the anatomical outcome after treatment of DME with anti-VEGF agents exists.^{12,13} To the best of our knowledge, the impact of long-term (>1 year) exposure to intravitreal anti-VEGF on microstructural alterations in DME has not yet been systematically evaluated.

Currently it is unclear, if an assumed additional deprivation of choroidal/retinal blood flow, because of long-term exposure to anti-VEGF (ranibizumab and/or aflibercept), leads to a deterioration of preexisting retinal microstructural alterations. It is further of interest whether decreasing IRF results into a regression of microstructural alterations as imaged by spectral-domain optical coherence tomography (SD-OCT) and whether different microstructural alterations are correlated with the functional outcome. Spectral-domain optical coherence tomography provides high-resolution noncontact imaging of the retinal substructures and allows for in vivo insight into the pathogenesis of DME.

Several studies revealed an association between functional outcomes and morphologic alterations as visualized by OCT (integrity of external limiting membrane [ELM], status of photoreceptor cone outer segment tips,¹⁴ disorganization of retinal inner layers

[DRIL],^{15,16} hyperreflective foci¹² and photoreceptor integrity,¹⁷ inter alia). Our study may elucidate whether detrimental or positive effects of anti-VEGF on preexisting microstructural alterations exist and therefore be of relevance for the future management of DME.

Material and Methods

This retrospective single-center study was conducted at the Department of Ophthalmology, City Hospital Triemli Zurich and was approved by the local ethics committee (Ethics Commission of the Canton Zurich, KEK-ZH-Nr. 2014-0601). Its conduction adhered to the tenets of the Declaration of Helsinki. Included patients gave their written informed consent on retrospective data evaluation and its publication. Data were retrieved from an internal database, containing clinical information of all patients treated with anti-VEGF agents during 2012 to 2016. In addition, medical charts and SD-OCT scans of included patients were reviewed.

Patients with a history of ≥ 20 intravitreal anti-VEGF injections (aflibercept and/or ranibizumab) for DME were included in the analysis. This results in an exposure period of ≥ 20 months, as the minimum injection interval was 1 month. At baseline (BL), patients were treatment naive regarding intravitreal anti-VEGF. End of study (EOS) was defined as the last available follow-up. All intravitreal injections (IVIs) were administered according to a treat-and-extend regimen. This protocol is characterized by the adjustment of individual injection intervals according to therapeutic response.¹⁸

Patients with further degenerative or vascular retinal diseases, ischemic maculopathy, vitreomacular traction syndromes, uncontrolled intraocular pressure, significant refractive media opacities, inflammatory ocular diseases, focal laser treatment and/or intravitreal steroid injections during the observation period were excluded from the analysis. Previous intravitreal steroid administration was not defined as exclusion criterion. Eyes with previous bevacizumab injections were excluded because of the lack of OCT data. Systemic data at BL (HbA1c, type and duration of diabetic disease) were obtained from existing medical records.

Spectral-domain optical coherence tomography images (Heidelberg Spectralis System, central foveal B-scan of 512 A-scans, $20^\circ \times 15^\circ$) were evaluated at BL (before first anti-VEGF injection) and after ≥ 20 IVI for DRIL, alterations at the level of the outer plexiform layer/Henle fiber layer

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junction (OPL/HE), integrity of external limiting membrane/ellipsoid zone (ELM/EZ), disruption of retinal pigment epithelium/Bruch complex (RPE/BR), retinal atrophy (RA), and maximal IRF height. Horizontal (DRIL, OPL/HE, ELM/EZ, RPE/BR, and RA) and vertical IRF extensions (μm) of microstructural alterations were manually measured in the horizontal central foveal scan using the integrated distance measurement tool of the Heidelberg Spectralis system. Two independent graders (ophthalmology residents with considerable experience in OCT reading) undertook measurements. Data of central retinal thickness (CRT) were extracted from the thickness map window of the computerized software of the Heidelberg Spectralis system. An integrated eye tracking system ensured the correct position for follow-up.

Primary outcome was the extent of retinal microstructural alterations (DRIL, OPL/HE, ELM/EZ, and RPE/BR) at BL versus after ≥ 20 IVI (i.e., EOS). Best-corrected visual acuity (BCVA) and degree of DME (CRT and IRF), as well as correlation analyses of microstructural alterations and BCVA were defined as secondary outcome measures.

For statistical purposes, BCVA measures (registered as Snellen acuity fractions) were converted into Early Treatment Diabetic Retinopathy Study scores as described by Gregori et al.¹⁹ Statistical analyses were performed using IBM SPSS statistics, version 22 for Microsoft Windows. Kolmogorov–Smirnov tests were conducted to test for data distribution. Wilcoxon signed rank tests and paired t-tests were used for asymmetrical (EML/EZ, RPE/BR, RA, BCVA, CRT, and IRF) and symmetrical distributions (DRIL and OPL/HE) of differences, respectively. Pearson correlation analyses, as well as a multivariate regression analysis (analysis of variance) were used to study correlations between BCVA and microstructural alterations. The impact of previous retinal laser coagulation on microstructural alterations was tested using independent samples Kruskal–Wallis tests. *P*-values < 0.05 were considered as statistical significant. The two independent graders were masked as to clinical data and to the number of injections. Interrater reliability was quantified by the intraclass correlation coefficient (two-sided, and mixed).

Results

Of 383 eyes screened, 37 eyes ($n = 37$) of 26 patients with DME met the inclusion criteria for the current retrospective analysis. The study group consisted of 16 male and 10 female patients. 29 eyes had non-proliferative and eight eyes proliferative diabetic retinopathy.

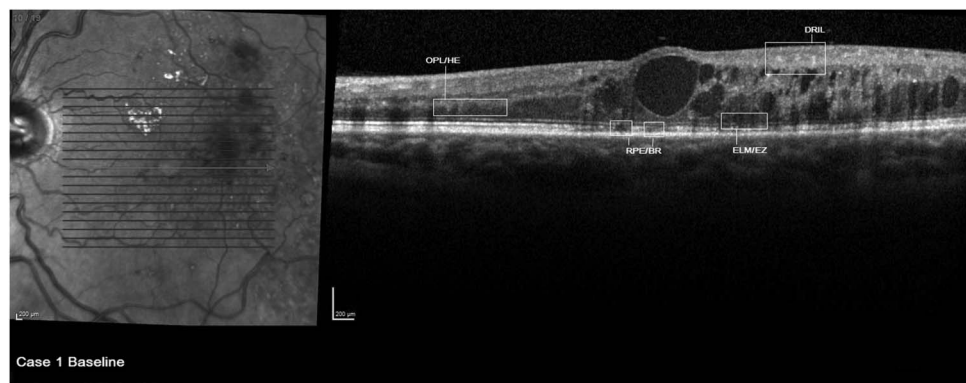
Mean age at BL amounted to 61.43 ± 9.8 years. Mean duration of systemic diabetic disease was indicated as 18.3 ± 9.1 years. Mean HbA1C at BL amounted to $7.1\% \pm 2.3$. Included eyes had a history of at least 20 IVI with aflibercept and/or ranibizumab. Included patients received a total of 1,069 injections (695 ranibizumab and 374 aflibercept). The treatment period with aflibercept and/or ranibizumab ranged from 21 to 65 months (mean = 42.84, SD ± 12.5 , equals 3.5 ± 1 year). The mean number of injections was 29.5 (SD ± 7.8). Four eyes (10.8%) were pretreated with other agents (intravitreal triamcinolone [$n = 3$] and/or dexamethasone [$n = 1$]), 1 patient with subcutaneous canakinumab ($n = 1$). Eighteen eyes (48.6%) underwent focal laser coagulation, 15 eyes (40.5%) panretinal laser coagulation before BL. See Table 1 for detailed patient characteristics.

At BL, all included patients had retinal microstructural alterations (DRIL 97.3%, $n = 36$; OPL/HE 100%, $n = 37$; ELM/EZ 64.9%, $n = 24$; RPE/BR 81.1%, $n = 30$; and RA 2.7%, $n = 1$). Figures 1 and 2 show microstructural alterations of a representative case at BL and EOS. With the exception of OPL/HE restoration, no significant change regarding retinal microstructural alterations was encountered between BL and EOS (i.e., ≥ 20 IVI). Detailed results are reported in Table 2. Central retinal thickness at BL amounted to $451.5 \pm 161.4 \mu\text{m}$ versus $288.7 \pm 51.1 \mu\text{m}$ at EOS ($P < 0.0001$). Intraretinal fluid height at BL amounted to $250 \pm 154.5 \mu\text{m}$ versus $69.1 \pm 73.4 \mu\text{m}$ at EOS ($P < 0.0001$). Mean BCVA significantly increased from 69.2 ± 11.6 letters at BL to 77.3 ± 7.4 at EOS ($P < 0.0001$). The extent of retinal microstructural alterations at BL was negatively correlated with BL

Table 1. Baseline Characteristics of Enrolled Patients

Age, years (mean \pm SD)	61.4 \pm 9.8
Sex, male/female, N	16/10
HbA1C % (mean \pm SD)	7.1 \pm 2.3
BCVA at BL (mean \pm SD letters early treatment diabetic retinopathy study)	69.2 \pm 11.6
Disease duration (years)	77.3 \pm 7.4
Diabetic retinopathy type, N	
Proliferative diabetic retinopathy	8
Nonproliferative diabetic retinopathy	29
Previous IVIs, N	
Triamcinolone	3
Dexamethasone	1
Canakinumab	1
Laser photocoagulation, N	
Panretinal	15
Focal	18

Fig. 1. Representative case with microstructural alterations on SD-OCT at BL.



BCVA (Pearson correlation analyses: DRIL $r = -0.362$, $P = 0.028$; OPL/HE $r = -0.452$, $P = 0.005$; ELM/EZ $r = -0.380$, $P = 0.020$; and RPE/BR $r = -0.429$, $P = 0.008$), as illustrated in Figure 3. In addition, a multiple regression was run to predict BCVA at BL from microstructural alterations (DRIL, OPL/HE, ELM/EZ, and RPE/BR). These variables statistically significantly predicted BCVA, $F_{4, 36} = 2.686$, $P = 0.049$, and $R^2 = 0.251$. Previous laser treatment had no effect on microstructural alterations. Independent samples Kruskal–Wallis tests did not reveal statistical significant differences between the three groups (no laser versus panretinal versus focal laser) at BL and EOS (BL DRIL $P = 0.294$; BL OPL/HE $P = 0.305$; BL ELM/EZ $P = 0.525$; BL RPE/BR $P = 0.676$; EOS DRIL $P = 0.699$; EOS OPL/HE $P = 0.346$; EOS ELM/EZ $P = 0.420$; and EOS RPE/BR $P = 0.511$).

Statistical analysis demonstrated an overall intra-class correlation coefficient among the different observers of 0.841 ± 0.12 (DRIL: 0.774 [95% confidence interval, CI: 0.56–0.88], OPL/HE: 0.834 [CI: 0.68–0.92], ELM/EZ: 0.908 [CI: 0.82–0.95], RPE/BR: 0.689 [CI: 0.39–0.84], and RA: 0.99 [CI: 0.98–0.99]), indicating good to excellent interobserver consistencies.²⁰

Discussion

Diabetic macular edema is highly prevalent and strongly associated with visual impairment. It therefore represents a condition of major medical and socioeconomic impact.²¹ Different and overlapping pathophysiologic mechanisms such as microvascular dysfunction with subsequent local inflammation and oxidative stress, as well as alterations at the neurovascular unit have been proposed.³ Recently, it was reported that neurodegenerative changes may precede clinically visible vascular changes.^{22,23}

Anti-vascular endothelial growth factor therapy currently represents the gold standard therapy leading to an amelioration of the vascular integrity, without significant effect on inflammatory or neuronal pathways.²⁴

Our data indicate that long-term intravitreal anti-VEGF (>20 months) therapy does not lead to a significant progression of preexisting microstructural alterations (DRIL, ELM/EZ, RPE/BR, and RA). Intravitreal aflibercept and/or ranibizumab seem to neither deteriorate nor have the capability to improve microstructural alterations (apart from CRT reduction and OPL/HE restoration). In contrast to Seo et al,¹³ who report about a continuous restoration of ELM/EZ integrity after ranibizumab treatment (follow-up of 12 months), we solely found

Fig. 2. Representative case with microstructural alterations after 21 intravitreal anti-VEGF injections.

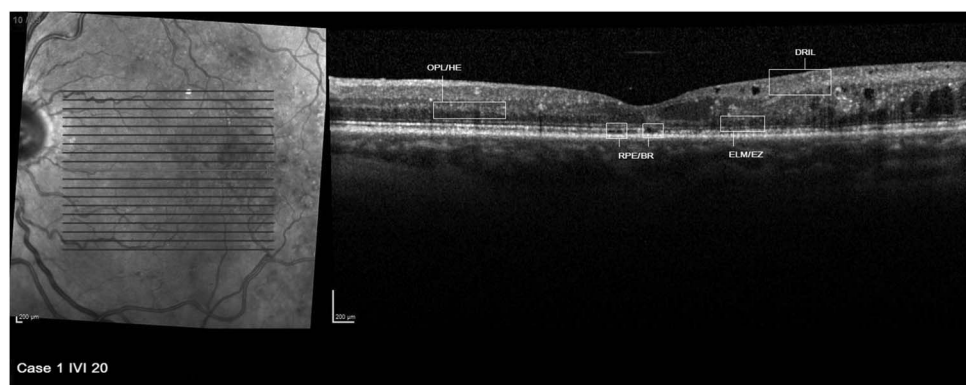


Table 2. Microstructural Alterations at BL Versus at EOS (After ≥ 20 Intravitreal Anti-VEGF Injections), *P*-values for Differences

Microstructural Alteration Type	BL (Mean \pm SD)	EOS (Mean \pm SD)	<i>P</i>
DRIL	2,228.5 \pm 1,023.1	2,093.22 \pm 958.0	0.381
OPL/HE	3,740.7 \pm 1,296.9	3,158.81 \pm 1,045.6	0.001
ELM/EZ	919.5 \pm 1,302.0	648.4 \pm 710.4	0.524
RPE/BR	1,063.2 \pm 1,065.0	864.4 \pm 721.9	0.122
RA	33.4 \pm 203.0	33.5 \pm 203.9	0.317

a restoration of OPL/HE layers. However, in the cited study, microstructural alterations have only been categorized (“well delineated” vs. “disrupted”) and measured centrally (central 1 mm). Paracentral or short segment alterations may therefore have been missed. Consistent with our results, Shin et al found no change of retinal nerve fiber layer thickness after multiple intravitreal anti-VEGF injections in the eyes with diabetic retinopathy.²⁵

Regarding the complex pathophysiology of DME and the herein involved pathways, VEGF blockage thus seems to solely play a minor role in the overall integrity of retinal microstructures. Further studies evaluating the impact of intravitreal steroids (involved in multiple pathways of disease development) on microstructural alterations compared with anti-VEGF would be of great interest. However, building on the theory of diabetic retinopathy as neurodegenerative disease, one would

rather expect degenerative neurosensory changes to be irreversible.

There is of course no doubt regarding the efficacy of anti-VEGF agents in IRF reduction and visual acuity gains; however, preexisting microstructural alterations should be taken into account once functional outcomes are predicted.

In agreement with previous studies,^{13,15,17} we found a correlation between BCVA and the extent of microstructural alterations. Microstructural alterations may serve as predictors for unfavorable functional outcome to a various extent. Our study did reveal a stronger correlation of outer microstructural alterations (i.e., RPE/BR, ELM/EZ, and OPL/HE) with BCVA than that of inner retinal layers (DRIL). This may be seen in the context of photoreceptor involvement and a presumed neuroplastic potential of neural circuits in retinal inner layers.

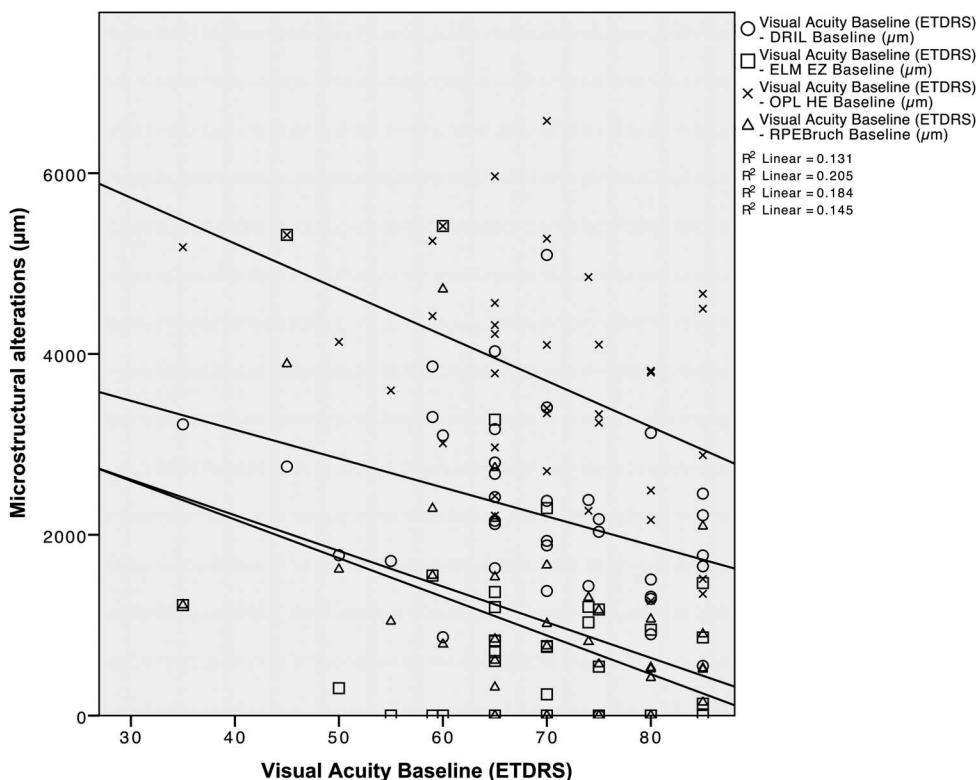


Fig. 3. Correlation of BCVA and microstructural alterations. Disorganization of retinal inner layers $r = -0.362$, $P = 0.028$; alterations at the level of the OPL/HE $r = -0.452$, $P = 0.005$; integrity of ELM/EZ $r = -0.380$, $P = 0.020$; disruption of RPE/BR $r = -0.429$, $P = 0.008$.

Previous laser treatment did not affect the size of microstructural alterations to a significant extent. This may be seen in the context, which no actual laser scars lay in the measured areas and that the 3 compared groups (no laser versus panretinal laser versus focal laser) were fairly small and inhomogeneously split.

Limitations

The small number of patients, the lack of a control group, and the retrospective study design should be mentioned as potential and important sources of bias. Optical coherence tomography measurements were not automated, and only central horizontal scans were evaluated. Graders did not undergo a specific training for OCT analysis. Image quality and presence of IRF influenced the accuracy of manual measurements to a certain degree.

Conclusion

In conclusion, intravitreal ranibizumab and aflibercept seems neither to have detrimental nor positive effects on microstructural alterations, with the known reduction of CRT but without relevant impact on restoration or promotion of retinal layer disruption. Alterations of the retinal microarchitecture are associated with unfavorable functional outcome in patients with DME and should be taken into account once visual acuity gains are predicted.

Key words: diabetic macular edema, vascular endothelial growth factor, ranibizumab, aflibercept, retinal layers, optical coherence tomography.

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